THE INHERITANCE OF ENVIRONMENTALLY INDUCED CHARACTERS IN BACTERIA

BY

JAMES MADISON SEVERENS

B. S., Michigan State College, 1935

M. S., University of Illinois, 1939

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BACTERIOLOGY IN THE GRADUATE SCHOOL OF THE UNIVERSITY OF ILLINOIS, 1943

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In the early days of bacteriology, views concerning variability of bacteria, were influenced greatly by Nägeli's concept of "pleomorphism". He believed that all species of bacteria were highly variable and capable of passing from one morphological state to another, as well as undergoing profound physiological changes. Furthermore, he claimed that one species could be the etiological agent in various diseases. This concept, however, was not without opposition. Cohn took the view that individual species preserved their morphological and other characters with great constancy. Much was done by Koch to establish Cohn's views upon a firm basis. His position in the bacteriological world and his methods of separating bacteria and obtaining pure cultures, enabled him to affirm that each transmissible disease was associated with its own specific microbe. He maintained the fixity and unalterable character of species, and even doubted that attentuation of virulence was due to other causes than contamination, or the death of a proportion of the individual organisms in a culture.

Koch's ideas gave rise to the theory of "monomorphism", which virtually denied the existence of variation in bacteria. Certain discrepancies in morphology and other characters were termed contamination or "involution forms". This theory persisted in bacteriology for many years and did much to hinder progress in the field of bacterial variation. It was too easily resorted to as an explanation and hindered investigators in determining the real facts. In spite of the influence of this concept, reports of variation, particularly as regards morphological characters, appeared in the literature. However, it was the work of Massini in 1907, which gave real impetus to studies of bacterial variation. Since that time it has become known that bacteria may vary in any of their characteristics.

It is now accepted as an established fact that bacteria are subject to variation, just as are all other forms of life. Differences of opinion, however, arose when attempts were made to explain its significance or its nature. Massini and the workers who immediately followed him, considered their variant forms, as

mutations. Later in 1918 and the years following, Löhnis, Enderlein, and Mellon formulated the life cycle theory. They regarded most variants which occurred in their cultures as stages in the development of bacteria, much in the same sense that insects undergo metamorphosis. Another viewpoint concerning the nature of bacterial variation, was introduced in 1920 with the work of Arkwright and the subsequent work of DeKruif and Hadley. These workers described the phenomenon, which is now known as "microbic dissociation". The word "dissociation" was introduced by DeKruif, and refers to the phenomenon by which definite species split up into a number of distinct forms, most, if not all of which are capable of returning to the normal without exceeding the bounds of the species. In recent years the dissociation concept and the life cycle theory have more or less joined forces, and the so-called dissociants are now looked upon by many as representing stages of development in a bacterial life cycle.

Within the last ten years, through the efforts of such men as Winge and Laustsen, Lindegren, Reed and others, attempts have been made to analyze bacterial variation in the light of modern genetic theory. It is the belief of these workers, that variation in bacteria differs in no fundamental way from variation in other forms of life. All well analyzed cases of hereditary differences in higher plants and animals have shown that the differences are due to Mendelian unit-characters or to combinations of these unit characters. Thus it is reasonable to assume that hereditary differences among bacteria may be similar in nature.

One of the important and interesting phases of bacterial variation is the phenomenon of "adaptation". That bacteria are able to become adapted to a wide range of environmental conditions is well known. Very little is known, however, about the process particularly as regards the following questions: (1) Can single cell cultures, therefore, genetically pure become adapted to different environments, merely by being subjected to them? (2) If so, what are the limits of their adaptive powers? (3) Are these induced characters permanent and transmissible to subsequent generations, or are they of a temporary nature? (4) What is the mechanism of adaption? Is it a result of selection of certain individuals in the population, or does the environment

act as a direct stimulus, producing changes in the cell, causing new characters to appear?

This investigation was designed with the purpose of obtaining some information which might prove useful in answering the above questions. Aside from the theoretical interest of the problem of adaptation, it is important from a practical standpoint, especially in the field of chemotherapy. In recent years, sulfonamide resistant forms of pathogenic bacteria, have become a problem in certain cases under treatment with one of these drugs.

The general procedure followed was to cultivate single cell cultures of bacteria in media containing increasing concentrations of a chemical, which exerted an inhibiting action upon the bacteria, until a tolerance or resistance to a given chemical was acquired. Following the acquisition of resistance, single cell cultures were isolated, subcultures of which were transferred to media free of chemical. These were tested at approximately thirty-day intervals to determine whether the newly acquired characters were transmitted to subsequent generations. Specificity of tolerance was determined by appropriate combinations of resistant strains and chemicals. Morphological and fermentative characters were observed during the course of the investigation.

Salmonella pullorum, Eberthella typhosa and *Salmonella schotmülleri* were the organisms used. The culture of *Salmonella pullorum* was obtained originally from the Animal Pathology Laboratory in 1936. The other species were obtained from the stock culture collection of the Department of Bacteriology. Plain beef extract broth has served as the media in all experiments. Single cell cultures were obtained by use of a Chambers' micromanipulator.

Results of the experiments showed that single cell cultures of *Salmonella pullorum, Eberthella typhosa* and *Salmonella schotmülleri* are able to adapt themselves to concentrations of sodium chloride, mercuric chloride and copper sulphate ranging from two and a half times to eight times the concentration which inhibited growth of the non-trained cultures. The resistant strains are specific in their ability to overcome inhibiting properties of each chemical. Strains resistant to the action of one chem-

ical are no more resistant to the action of another chemical than is the non-adapted strain. This specificity proves that resistant strains are not merely hardy vigorous races capable of growing under adverse conditions, but that the change is effective only in regard to one particular chemical.

That these changes were hereditary in nature was shown by the fact, that resistant strains retained their resistance throughout the course of experimentation, a period of eighteen months, during which time fifty-five transfers were made. Even at the end of this period there was no indication of decrease in the ability to resist the action of the chemicals. During the investigation only very slight changes occurred in the cell morphology, while the fermentative characters of the resistant strains remained unchanged.

The results of a series of experiments showed that normal populations of the bacteria studied, contained a few individual cells which possessed the ability to withstand the action of the chemicals without a previous training period. It is not yet certain whether these mutant resistant forms were already present in the normal population, or whether a few cells were susceptible to change by the action of a particular chemical.

In view of the hereditary nature of the changes and their specificity, it seems logical to classify them as mutations. This view is further supported by the isolation of the resistant cells from a normal population. In conclusion it is suggested that the adaptation process is likely a selection of certain individuals from the population, which are better able to survive under a given condition. Prolonged cultivation in the presence of certain environmental factors, will thus lead to a population composed entirely of the mutant form.

BIBLIOGRAPHY

Arkwright, J. A., 1920. Variation in bacteria in relation to agglutination by salts and by specific sera. J. Path. and Bact. 23:358-360.

Cohn, F. 1875. Untersuchungen uber Bacterien. Beitr. Biol. Pfl. Breslau i: 127-224.

DeKruif, P. 1921. Dissociation of microbic species. I. Mutation in pure line strains of the bacillus of rabbit septicemia. Proc. Soc. Exp. Biol. and Med. 19:34-37.

Enderlein, G. 1925. Bakterien-Cyclogenie.

Hadley, P. 1927. Microbic dissociation. J. Inf. Dis. 40: 1-312.

Koch, R. 1881. See Löhnis 1921. Mem. Nat. Acad. Sci. 16: 1-252.

Lindegren, C. C. 1935. Genetical studies of bacteria. II. The problem of bacterial variation. Cent. Bakt. II Abt. 93: 113-122.

Löhnis, F. 1921. Studies upon the life cycles of the bacteria. Mem. Nat. Acad. Sci. 16: 1-252.

Massini, R. 1907. Ueber einen in biologischer Beziehung interessantere Kolistamm. Arch. Hyg. 61: 250-292.

Mellon, R. R. 1925. The sexual cycle of B. coli in relation to the origin of variants with special reference to Neisser's and Massini's B. coli mutabile. J. Bact. 10:579-586.

Nageli, C. V. 1877. Cited by Hadley, 1927.

Reed, G. B. 1937. Independent variation of several characteristics in S. marsescens. J. Bact. 34: 255-260.

Winge, O. and Laustsen, O. 1939. The breeding of new yeast types through hybridization. The Baker's Tech. Digest, 13: 123-125. See also Comptes rendus d. Lab. Carlsberg, Ser. physiol. 21, 77, 1935; ibidem 22, 235-244, 1938.

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Publications are as follows:

1. C. S. Bryan, O. J. Robinson, and J. M. Severens
   Home pasteurization of Streptococcus-infected milk. Mich. Ag. Exp. Sta. Quart. Bull. Vol. 19, No. 1, 1936.
2. E. Roberts, J. M. Severens, and L. E. Card
   Nature of the hereditary factors for resistance and susceptibility to pullorum disease in the domestic fowl. Proc. Seventh World's Poultry Congress, 1939.
3. E. Roberts, J. M. Severens, and L. E. Card
   Effect of environment on the expression of resistance and susceptibility to disease in the domestic fowl. Proc. Seventh World's Poultry Congress, 1939.
4. B. R. Burmester, J. M. Severens, and E. Roberts
   Blood cells in the bone marrow of the chick before and after hatching. Poultry Science XX, No. 5, 1941.